Motility of live cancer cells quantified by Fourier phase microscopy

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Abstract: Using Fourier phase microscopy, the motility of epithelial cancer cells has been quantified. The mean squared displacement analysis suggest that the cell motion is superdiffusive for cells at various stages of their life cycle.

OCIS codes: (180.0180) Microscopy, (170.1530) Cell analysis, (170.6920) Time-resolved imaging;

1. Introduction

The quantitative analysis of motility associated with cells in culture provides the necessary framework for modeling dynamics and testing specific hypotheses [1, 2]. In particular, the statistical investigation of the center of mass trajectories of live cells provides information about the nature of the motion and significant scaling parameters involved.

Various microscopy techniques, such as bright field [3], phase contrast, and Nomarski/ DIC [4, 5], have been used to track the centroid of live cells. More recently, fluorescence microscopy has been used to study the motion of hydra cells [6] and lymphocytes [7]. However, information provided by these techniques is qualitative in terms of the mass distribution of cells, inasmuch as the intensity-weighted centroid of the cell does not necessarily overlap with the actual center of mass. On the other hand, it has been shown some time ago that the optical phase shift through the cells is linearly proportional to the dry mass of the biological structure [8].

Recently, Fourier Phase Microscopy (FPM) has been demonstrated as a novel technique for retrieving quantitative phase images with high transverse resolution and sub-nanometer path-length stability over several hours [9]. In this paper we show that due to its remarkable stability, FPM is suitable for investigating cellular dynamics over extended periods of time. Our experiments, performed on HeLa cells in culture, and the analysis in terms of mean squared displacements, reveal significant differences in motility between mitotic and non-mitotic cells. To our knowledge, this is the first time that quantitative phase imaging has been used to quantify cellular motility. Additionally, the relationship between mitosis stage and cell motility has not been measured previously.

2. Fourier Phase Microscopy (FPM)

FPM is capable of retrieving the two-dimensional phase distribution of a polychromatic field with high accuracy and low noise; its principle is described in reference [9].

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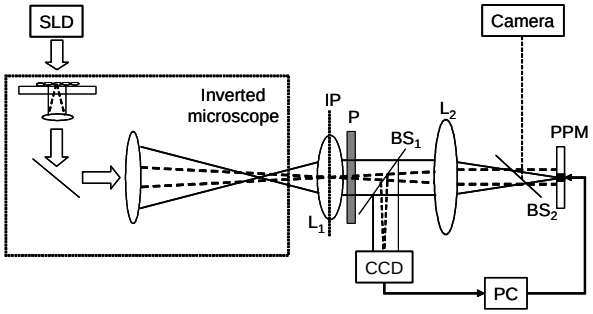

Figure 1. Experimental setup. SLD superluminescent diode; L_1 corrective lens; IP image plane; P polarizer; BS beam splitter; L_2 Fourier lens; PPM programmable phase modulator; CCD charge-coupled device.

The experimental setup is depicted in Fig. 1. The low coherence field from a superluminescent diode (center wavelength 809 nm, bandwidth 20 nm) is used as the illumination source for an inverted microscope. The light is coupled into a single mode fiber and subsequently collimated, such that essentially plane wave illumination of the sample was achieved in the transmission geometry. Through the video port, the microscope produces a magnified image positioned at the image plane IP. The lens L_1 is positioned at the same plane IP and has a focal length such that it collimates the zero-spatial frequency field (solid line in Fig. 1). Thus, the image field formed by L_1 has the same position and magnification as the microscope image and can now be Fourier transformed as in standard 4-f geometries. The Fourier transform of the image field is projected by the lens L_2 (50 cm focal length) onto the surface of a programmable phase modulator (PPM) (Hamamatsu KK Photonics, model X8267). The PPM consists of an optically addressed, two-dimensional liquid crystal array with 768x768 active pixels. The polarizer P adjusts the field polarization in a direction parallel to the molecular axis of the liquid crystal. In this configuration, the PPM produces precise control over the phase of the light reflected by its surface. The PPM pixel size is 26x26 μm^2, while the dynamic range of the phase control is 8 bits over 2π. In the absence of PPM modulation, an exact phase and amplitude replica of the image field is formed at the CCD plane via the beam splitter BS_1. For alignment purposes, a camera is used to image the surface of the PPM via the beam splitter BS_2. The PPM is used to controllably shift the phase of the spatially varying field component V_1 (solid line in Fig. 1) in 4 increments of $\pi/2$ with respect to the average field V_0 (dotted line), as in typical phase-shifting interferometry measurements [10]. This procedure allows retrieval of the full-field phase information associated with the sample [9]. In our studies the acquisition rate was limited due to the 8 Hz refresh rate of the PPM liquid crystal. The acquisition rate is not limited in principle and can be increased using a faster phase shifter.

3. FPM investigation of cell motility

The motility of mitotic and non-mitotic cells has been investigated by statistically analyzing their center of mass positions. Prior to physically separating into two sister cells (cytokinesis), mitotic cells round up and spend 30-60 minutes in this shape, while the chromatin content is essentially doubling. In order to quantitatively compare the motility of mitotic vs. non-mitotic cells, we considered the mitotic cells only during the time that their ellipticity exceeded 0.9, i.e. when their shape was roughly circular. The cell motility during this period of the life cycle was compared with that of cells that did not undergo any mitotic activity during the time of observation. Figure 2(c) shows an example of the center of mass trajectories for the non-mitotic and mitotic cells shown in 2(a) and 2(b), respectively. The trajectory of the non-mitotic cell is significantly more confined in space, indicating stronger attaching forces to the substrate surface.

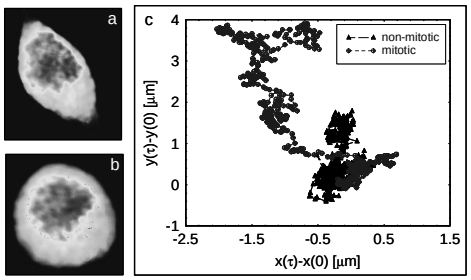

Figure 2. FPM image of a (a) non-mitotic and (b) mitotic HeLa cell. (c) Respective center of mass trajectories, as indicated.

To obtain a statistical comparison between the motility of mitotic and non-mitotic cells, the center of mass positions of many cells were recorded. These displacements were used to calculate the mean squared displacements $\left\langle \Delta r(\tau)^2 \right\rangle$ associated with the two types of cells. The number of cells investigated in a given field of view varied from approximately 5 to 40, depending on the magnification. A total of 15,000 cell steps were recorded, and the results in terms of mean squared displacements summarized. Remarkably, $\left\langle \Delta r(\tau)^2 \right\rangle$ at long times approaches a power law dependence. This behavior is more apparent for the non-mitotic cells, which can be characterized by a power law function with an exponent of 1.25. This is a clear indication of the superdiffusive motion of the live cells. In addition, the mean squared displacement of the mitotic cell at long times is approximately a factor of 2 higher than that of the non-mitotic cells, which demonstrates once more the increased motility of cells during the metaphase of mitosis.

Such power law behavior suggests that the trajectory patterns obey self similar scaling properties. Superdiffusive cell motion has been also observed in hydra cells, and Tsalis statistics has been proposed to model such behavior [11]. To our knowledge, this is the first time that a statistical analysis has been performed on cells during different stages of their life cycle.

4. References

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